CERTIFICATE OF TRANSLATION

I Roger P. Lewis, whose address is 42 Bird Street North, Martinsburg WV 25401, declare and state the following:

I am well acquainted with the English and Japanese languages and have in the past translated numerous English/Japanese documents of legal and/or technical content.

I hereby certify that the Japanese translation of the attached translation of document identified as:

Olympus Patent Application Reference No. 03P00542

"Magnifying video system, endoscope for *in vivo* cellular observation, and *in vivo* cellular observation method"

is to the best of my knowledge and ability true and accurate.

I further declare that all statements contained herein of our own knowledge, are true, that all statements of information and belief are believed to be true.

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[Title of the invention]

Magnifying video system, endoscope for *in vivo* cellular observation, and *in vivo* cellular observation method.

[Claims]

[Claim 1]

A video system provided with a video module comprising an objective optical system having a scale factor larger than 1 and an illumination optical system with a light source for supplying illumination light, characterized by the fact that:

the light source is provided with a wavelength selection means for dividing, among the blue, green, and red wave ranges in the illumination light, the blue or red wave range into two wave bands T1 and T2 and cutting off the wave band T1 closer to the green wave range.

[Claim 2]

The video system according to Claim 1, characterized by the fact that the wave band T1 ranges between 600nm and 700nm.

[Claim 3]

An endoscope provided with an observation unit and an illumination unit, characterized by the fact that:

the observation unit comprises an objective optical system and the observation view field of the objective optical system at the in-focus position is apart from the illuminating field of the illumination unit.

[Claim 4]

An endoscope provided with a video module comprising an objective optical system having a scale factor larger than 1, characterized by the fact that:

the endoscope is provided with an illumination unit and the video module and illumination unit are disposed in a manner in which the observation view field of the video module at the in-focus position does not overlap

the illuminating field of the illumination unit.

[Claim 5]

An observation method in which a first illumination means and a second illumination means facing the first illumination means are used to illuminate the observation view field for an object placed between the first and second illumination means, characterized by comprising a step in which the second illumination means scatters the illumination light from the first illumination means to illuminate the observation view field.

[Claim 6]

The observation method according to Claim 5 characterized by further comprising a step in which the first illumination means emits the illumination light toward the second illumination means from outside the observation view field of an object.

[Claim 7]

A method for observing epithelial cells of a living tissue using the endoscope according to Claim 3, characterized by comprising a step in which the illumination light emitted from the illumination system is scattered and reflected by the parenchymal tissues underlying the epithelial cells to illuminate them.

[Claim 8]

A method for observing epithelial cells of a living tissue using the endoscope according to Claim 4, characterized by comprising:

A step in which the illumination system of the endoscope illuminates the underlying parenchymal tissues of the epithelial cells; and

a step in which the illumination light emitted from the illumination system is scattered and reflected by the parenchymal tissues underlying the epithelial cells to illuminate them.

[Claim 9]

An *in vivo* cellular observation method in which, based on magnified images of a living tissue, the number of cell nuclei captured in the view field is used to evaluate the cell size or the distance between cell nuclei

captured in the view field is used to evaluate the nucleus density for diagnosis of abnormal cells, characterized by comprising the following steps:

introducing a coloring agent having blue or red wavelength absorbency into cells of a living tissue to be imaged;

illuminating the cells with light having the absorption wavelength;

enhancing the contrast of cell nuclei using the difference in retention rate of the coloring agent between the nuclei and remaining portions of cells; and

displaying several tens to several hundreds of cell nuclei captured in the view field.

[Claim 10]

An *in vivo* cellular observation method in which, based on magnified images of a living tissue obtained by the video system according to Claim 1, the number of cell nuclei captured in the view field is used to evaluate the cell size or the distance between cell nuclei captured in the view field is used to evaluate the nucleus density for diagnosis of abnormal cells, characterized by comprising the following steps:

applying blue or read wave band absorbency to cells of a living tissue to be imaged;

dividing the absorption wave band into two wave bands T1 and T2 and illuminating the cells with the illumination light from which the wave band T1 closer to the green wave range is cut off;

enhancing the contrast of cell nuclei using the difference in light absorbance for the wave band T2 between the nuclei and remaining portions of cells; and

displaying several tens to several hundreds of cell nuclei captured in the view field.

[Claim 11]

An *in vivo* cellular observation method in which, based on magnified images of a living tissue, the occupancy of cell nuclei within the cell walls of cells captured in the view field is evaluated for diagnosis of abnormal cells, characterized by comprising the following steps:

introducing a coloring agent having blue or red wavelength absorbency into cells of a living tissue to be imaged;

illuminating the cells with light having the absorption wavelength;

enhancing the contrast of cell nuclei using the difference in the retention rate of the coloring agent between the nuclei and remaining portions of cells; and

displaying several cell nuclei captured in the view field.

[Claim 12]

An *in vivo* cellular observation method in which, based on magnified images of a living tissue obtained by the video system according to Claim 1, the occupancy of cell nuclei within the cell walls of cells captured in the view field is evaluated for diagnosis of abnormal cells, characterized by comprising the following steps:

applying blue or read wave band absorbency to cells of a living tissue to be imaged;

dividing the absorption wave band into two wave bands T1 and T2 and illuminating the cells with the illumination light from which the wave band T1 closer to the green wave range is cut off;

enhancing the contrast of cell nuclei using the difference in light absorbance for the wave band T2 between the nuclei and remaining portions of cells; and

displaying several cell nuclei captured in the view field.

[Claim 13]

The *in vivo* cellular observation method according to Claim 11 characterized by the fact that:

an endoscope is used to obtain magnified images of a living tissue and the endoscope is provided with an objective optical system having a numerical number on the object side of 0.3 or larger.

[Claim 14]

The *in vivo* cellular observation method according to Claim 12 characterized by the fact that:

the objective optical system of the video module used for observation has a numerical number on the object side of 0.3 or larger.

[Claim 15]

An endoscope characterized by being used in the observation method according to Claim 5.

[Claim 16]

An endoscope characterized by being used in the observation method according to Claim 6.

[Claim 17]

An endoscope characterized by being used in the *in vivo* cellular observation method according to Claim 9.

[Claim 18]

An endoscope characterized by being used in the *in vivo* cellular observation method according to Claim 11.

[Detailed explanation of the invention]

[0001]

[Scope of the invention]

The present invention relates to a magnifying video system suitable for *in vivo* cellular observation, an endoscope for *in vivo* cellular observation, and an *in vivo* cellular observation method.

[0002]

[Prior art technology]

Conventional endoscopes have a large field angle between 90° and 140° so that tissues inside the body can be observed without overlooking lesions.

[0003]

Conventional endoscopes change the distance to the object in order to obtain magnified or reduced images of an object to be observed, and thus have a large field depth for a fixed focal point so that objects at a distance between 3mm and 50mm can be observed without focusing operations.

[0004]

Conventional endoscopes as those described above have an image scale factor of approximately 30 to 50 on a 14 inch screen monitor, which is sufficient to observe diseased tissues.

Zoom optical systems are used with conventional endoscopes to obtain further magnified images. The largest scale factor of zoom optical systems is approximately 70 on a 14 inch screen monitor. The zoom optical system has a built-in zoom lens driving mechanism. As a result, the endoscope has an insert tip with an outer diameter larger than 10mm and requires complex operations. Such endoscopes have limited applications.

[0005]

Living tissues to be observed by the conventional endoscope and how they can be observed is described hereafter with reference to Fig.1. Living tissues to be observed by the conventional endoscope consists of vascular parenchymal tissues and underlying transparent epithelial cells. Incident light illumination emitted from above by the endoscope reaches the parenchymal tissues via the transparent epithelial cells and is scattered there (A1, A2 in the figure). Most of the light re-enters the epithelial cells. The illumination light is diffracted by the cell walls and nuclei when it is transmitted through the epithelial cells (B1, B2 in the figure). The diffracted light is weak and the light scattered by the parenchymal tissues is dominant.

Consequently, only the parenchymal tissues are observed through an objective optical system in a conventional endoscope.

[0006]

When diagnosis of abnormality is difficult to make by the observation of tissue images, such as in a case a lesion is very small, a suspicious tissue is taken using a therapeutic instrument in the course of endoscopy. The tissue taken is cellularly examined under microscopy. The endoscope uses incident light illumination from an illumination optical system disposed around the objective optical system. The microscope uses the objective optical system and illumination optical system on either side of the sample with the sample being illuminated from the back, i.e. transmission light illumination. The sample is pre-processed to make it suitable for observation, such as by removing the parenchymal tissues and thin slicing for reduced scattering and better light transmission and/or staining for contrast.

[0007]

How the sample is observed is described hereafter with reference to Fig.2. The prepared sample is fixed on a cover glass and illuminated from below. The illumination light is diffracted by the cell walls and nuclei when it is transmitted through the sample. The diffracted lights interfere with one another to enhance or attenuate themselves, producing contrast. Thus, the objective optical system placed above can observe the sample.

[0008]

Laser scanning type confocal endoscopes have been proposed that can be inserted in the body and have resolution sufficient for cellular observation. The confocal optical system has a pinhole such as an airy disk at the conjugate position to the image plane and acquires information of a diffraction limit level for each point of an object in the view field. A laser beam from a light emitting optical system scans the object and information obtained from the reflected light from the object for each point is combined to produce an image representing two- or three-dimensional information. High resolution is realized not only crosswise in the plane but also in the depth direction.

[0009]

[Problems overcome by the invention]

It takes several days to several weeks to identify abnormal tissue in the conventional manner in which living tissues are removed and examined *ex vivo*. In addition, the cellular sample to be observed is a tiny part of the removed tissue that is isolated and fixed. It will provide information on cellular structures, but no

functional information such as the fluid circulation within cells because of the completely different circumstances from *in vivo*. Thus, there is a need to magnify endoscopes that allow real-time *in vivo* observation of intact living cells.

[0010]

A small-sized video module provided with an objective optical system having a high image scale factor competitive to a microscope and with high resolution is necessary to form the cellular images of a lesion in the body. The objective optical system used in a conventional endoscope does not meet these requirements. In a conventional endoscope, as described with reference to Fig.1, the illumination light is diffracted by the cell walls and nuclei when it is transmitted through the epithelial cells (B1, B2 in the figure). The diffracted light is weak and light scattered by the parenchymal tissues is dominant (A1, A2 in the figure). Consequently, the data from the epithelial cells are submerged and only the data from the parenchymal tissues are imaged through an objective optical system in a conventional endoscope.

[0011]

The objective optical system used in microscopes is satisfactory in performance, but is large and difficult to insert hardly into the living body. The laser scanning type confocal endoscope has a problem with scanning speed and is still immature for real-time *in vivo* observation.

[0012]

As described above, a video system that meets the requirements for *in vivo* cellular observation has so far not been realized.

The purpose of the present invention is to solve the above problems and to provide a video system for realizing real-time *in vivo* cellular observation, an *in vivo* cellular observation method using the video system, and an endoscope for *in vivo* cellular observation.

[0013]

[Problem resolution means]

In order to solve the problems above, the video system according to the present invention has the following characteristics:

(1) A video system provided with a video module comprising an objective optical system having a scale

factor larger than 1 and an illumination optical system and with a light source for supplying illumination light, characterized by the fact that:

the light source is provided with a wavelength selection means for dividing, among the blue, green, and red wave ranges in the illumination light, the blue or red wave range into two wave bands T1 and T2 and cutting off the wave band T1 closer to the green wave range.

(2) The video system according to (1), characterized by the fact that the wave band T1 ranges between 600nm and 700nm.

[0014]

In order to solve the above problems, the endoscope according to the present invention has the following characteristics.

(3) An endoscope provided with an observation unit and an illumination unit, characterized by the fact that:

the observation unit comprises an objective optical system and the observation view field of the objective optical system at the in-focus position is apart from the illuminating field of the illumination unit.

(4) An endoscope provided with a video module comprising an objective optical system having a scale factor larger than 1, characterized by the fact that:

the endoscope is provided with an illumination unit and the video module and illumination unit are disposed in the manner that the observation view field of the video module at the in-focus position does not overlap the illuminating field of the illumination unit.

[0015]

In order to solve the problems above, the *in vivo* cellular observation method according to the present invention has the following characteristics.

(5) An observation method in which a first illumination means and a second illumination means facing the first illumination means are used to illuminate the observation view field of an object placed between the first and second illumination means, characterized by comprising the step in which the second illumination means scatters the illumination light from the first illumination means to illuminate the observation view field.

(6) The observation method according to (5) characterized by further comprising a step in which the first illumination means emits the illumination light to the second illumination means from outside the observation view field of an object.

(7) A method for observing epithelial cells of a living tissue using the endoscope according to (3), characterized by comprising the step in which the illumination light emitted from the illumination system is scattered and reflected by the parenchymal tissues underlying the epithelial cells to illuminate them.

(8) A method for observing epithelial cells of a living tissue using the endoscope according to (4) characterized by comprising:

the step in which the illumination system of the endoscope illuminates the parenchymal tissues underlying the epithelial cells; and

the step in which the illumination light emitted from the illumination system is scattered and reflected by the parenchymal tissues underlying the epithelial cells to illuminate them.

(9) An *in vivo* cellular observation method in which, based on the magnified images of a living tissue, the number of cell nuclei captured in the view field is used to evaluate the cell size or the distance between cell nuclei captured in the view field is used to evaluate the nucleus density for diagnosis of abnormal cells, characterized by comprising the following steps:

introducing a coloring agent having blue or red wavelength absorbency into cells of a living tissue to be imaged,

illuminating the cells with light having the absorption wavelength;

enhancing the contrast of cell nuclei using the difference in the retention rate of the coloring agent between the nuclei and remaining portions of cells; and

displaying several tens to several hundreds of cell nuclei captured in the view field.

(10) An *in vivo* cellular observation method in which, based on the magnified images of a living tissue obtained by the video system according to (1), the number of cell nuclei captured in the view field is used to evaluate the cell size or the distance between cell nuclei captured in the view field is used to evaluate the nucleus density for diagnosis of abnormal cells, characterized by comprising the following steps:

applying blue or read wave band absorbency to cells of a living tissue to be imaged;

dividing the absorption wave band into two wave bands T1 and T2 and illuminating the cells with the illumination light from which the wave band T1 closer to the green wave range is cut off;

enhancing the contrast of cell nuclei using the difference in light absorbance for the wave band T2 between the nuclei and remaining portions of cells; and

displaying several tens to several hundreds of cell nuclei captured in the view field.

(11) An *in vivo* cellular observation method in which, based on magnified images of a living tissue, the occupancy of cell nuclei within the cell walls of cells captured in the view field is evaluated for diagnosis of abnormal cells, characterized by comprising the following steps:

introducing a coloring agent having blue or red wave band absorbency into cells of a living tissue to be imaged;

illuminating the cells with light including the absorption wave band;

enhancing the contrast of cell nuclei using the difference in retention rate of the coloring agent between the nuclei and remaining portions of cells; and

displaying several cell nuclei captured in the view field.

(12) An *in vivo* cellular observation method in which, based on magnified images of a living tissue obtained by the video system according to (1), the occupancy of cell nuclei within the cell walls of cells captured in the view field is evaluated for diagnosis of abnormal cells, characterized by comprising the following steps:

applying blue or read wave band absorbency to cells of a living tissue to be imaged;

dividing the absorption wave band into two wave bands T1 and T2 and illuminating the cells with the illumination light from which the wave band T1 closer to the green wave range is cut off;

enhancing the contrast of cell nuclei using the difference in light absorbance for the wave band T2 between the nuclei and remaining portions of cells; and

displaying several cell nuclei captured in the view field.

(13) The *in vivo* cellular observation method according to (11) characterized by the fact that:

an endoscope is used to obtain magnified images of a living tissue and the endoscope is provided with an objective optical system having a numerical number on the object side of 0.3 or larger.

(14) The *in vivo* cellular observation method according to (12) characterized by the fact that:
the objective optical system of the video module used for observation has a numerical number on the object side of 0.3 or larger.

[0016]

In order to solve the problems above, the endoscope according to the present invention has the following characteristics.

(15) An endoscope characterized by being used in the observation method according to (5).

(16) An endoscope characterized by being used in the observation method according to (6).

(17) An endoscope characterized by being used in the *in vivo* cellular observation method according to (9).

(18) An endoscope characterized by being used in the *in vivo* cellular observation method according to (11).

[0017]

[Embodiments]

The magnifying video system according to the present invention applied to an endoscope for *in vivo* cellular observation is described hereafter.

[0018]

First, a conventional endoscope having a wide view field is used for thorough examination of diseased tissues in the body without overlooking. For a region that is difficult to diagnose from the tissue image from a conventional endoscope, an endoscope to which the magnifying video system of the present invention is applied (hereafter termed the magnifying endoscope) is used to cellularly examine the region.

[0019]

For magnified cellular observation, coloring agents are previously delivered if necessary. A certain time period after the coloring agent is delivered, the difference in time required for the cell nuclei, wall, and others to excrete the coloring agent create contrast in the object. Then, the magnifying endoscope is guided to the region in question and contact made with the object at the tip while the observation through the conventional endoscope is continued. Preferably, a tissue image from the conventional endoscope and a cell image from the magnifying endoscope are both simultaneously displayed on a TV monitor. In this way, the magnifying endoscope can be guided precisely to a very small targeted region within an extensive observation view field to magnify and observe cell nuclei and walls.

[0020]

Before the detailed explanation of the magnifying video system according to the present invention, the requirements for a video module used in the magnifying video system are discussed.

First, the scale factor required for visualizing fine cellular structures is discussed. The observation scale factor βm on a monitor is given by the following expression:

[0021]

$$\beta m = \beta o * \beta d$$

in which βo is the scale factor of the objective optical system, which is the scale factor at which an object image is formed on the image pick-up element. βd is the display scale factor, resulting from the monitor display screen size divided by the image pick-up element display screen size.

[0022]

Conventional endoscopes realize a scale factor of 30 to 50 on a 14 inch monitor. Zoom optical systems having a magnifying function realize a scale factor of approximately 70. An observation scale factor of approximately 200 to 2000 is necessary for cellular observation on a 14 inch monitor. Therefore, it is desired that the objective optical system satisfies the following conditions:

[0023]

$$1 < |\beta o| \le 10 \ldots (1)$$

in which βo is the scale factor of the objective optical system;

$$0.9 \leq | \cos wy' / \cos wy | \leq 1.1 \ldots (3)$$

in which wy' is the incident angle at which the main ray corresponding to the largest field angle enters the image pick-up surface and wy is half the field angle. When the conditional expression exceeds the lower limit, the incident angle to the image pick-up element will be larger, failing to maintain uniform image qualities (for example, color reproducibility and brightness) within the view field. When this conditional expression exceeds the upper limit, the field angle will be larger, failing to ensure a required scale factor.

[0024]

The resolution is discussed hereafter.

Diseased tissues can be identified with a resolution of millimeters or sub-millimeters. Cellular observation requires a resolution of microns or sub-microns. In order to form detailed images of an object that is transparent and has little difference in refractive index and a low contrast, the interference of diffracted lights from the object is used to enhance the contrast. The objective optical system has to have a larger numerical aperture NA on the object side so as to collect higher degrees of diffracted lights and preferably satisfies the following condition:

[0025]

$$0.1 \leq NA \leq 0.8 \ldots (4)$$

In addition, in order to obtain both high contrast and fineness, the objective optical system has to have resolution higher than that determined by the pitch of the image pick-up element and lower than that determined by the diffraction limit and preferably satisfies the following condition:

[0026]

$$0.1 \leq 0.61 * \lambda * p / \beta o \leq 0.8 \ldots (6)$$

in which p is the pixel size of the image pick-up element, λ is the e line wavelength 0.546 [μm], and βo is the scale factor of the objective optical system. When the conditional expression exceeds the lower limit, sufficient contrasts will not be obtained. When the conditional expression exceeds the upper limit, aberrations become difficult to correct, failing to obtain fine images.

[0027]

Down-sizing is discussed hereafter.

It is desired that the magnifying endoscope has an outer diameter of less than ϕ4mm in order to guide it to an object through the treatment tool insert channel of a conventional endoscope. Accordingly, it is desired that the objective optical system be down-sized to have an outer diameter of less than ϕ2mm.

[0028]

A desired small-sized objective optical system having a high image scale factor and a high resolution comprises, from the object side, a lens group having a positive focal length and an aperture diaphragm and satisfies the following condition:

[0029]

$$0.2 \leq \phi 1 / (\phi 2 / f1) \leq 2 \ldots (5)$$

in which $\phi 1$ is the numerical aperture of the aperture diaphragm, $\phi 2$ is the largest outer diameter of the objective optical system, and f1 is the focal length of the lens group having a positive focal length.

[0030]

This conditional expression prevents the objective optical system from having a larger aperture in association with a larger NA, this is to say, to achieve down-sizing. When the conditional expression exceeds the lower limit, the objective optical system will have a larger total length and a larger outer diameter, hampering down-sizing. When the conditional expression exceeds the upper limit, aberrations will be difficult to correct.

[0031]

It is desired that the objective optical system comprises, from the object side, a front group having a positive focal length, an aperture diaphragm, and a rear group having a positive focal length in order to obtain a view field with no curvatures up to the periphery thereof. In such a case, the following condition is desirably satisfied to achieve both down-sizing and a high image scale factor:

[0032]

$2 \leq f2 / f1 \leq 10$ ... (2)

in which f1 is the focal length of the front group and f2 is the focal length of the rear group. When the conditional expression exceeds the lower limit, a required image scale factor will not be endured. When the conditional expression exceeds the upper limit, it will lead to a larger total length and a larger outer diameter, hampering down-sizing.

[0033]

Embodiments of the video module of the present invention are described hereafter.

(Embodiment 1)

Fig.11 is a cross section of the lens of Embodiment 1 and Table 1 provides the lens data.

[0034]

The structure of Embodiment 1 is described hereafter with reference to Fig.11 (a).

The objective unit comprises an objective lens group 101 having a uniform diameter in an objective frame 102. The objective lens group 101 consists of, from the object side, a first group having a positive focal length, an aperture diaphragm 103, and a second group having a positive focal length. An image pick-up element 105 is fixed to an image pick-up frame 106 via a cover glass 104, forming an image pick-up unit.

[0035]

The video module is focused by changing the distance 107 between the objective unit and image pick-up unit. The insert section for the magnifying endoscope is constructed by a hard tip member 108 and an outer sheath member 110. The video module is fixed into the insert section via an intermediate member 109.

[0036]

Fig.11 (b) is a cross section seen in the direction indicated by the arrow A in Fig.11 (a). The intermediate member 109 has cutouts (shaded parts in the figure) on the perimeter, through which an illumination fiber 111 is inserted and fixed. After the intermediate member 109 and illumination fiber are fixed to the hard tip member 108, the video module is inserted and fixed.

[0037]

When adjustment is required for example in image scale factor, gap adjustment members 112a and 112b provided before and after the aperture diaphragm 103 can be moved for larger or smaller space if necessary. Gap adjustment rings made of ultra-thin plate is used for gap adjustment. The gap adjustment part is designed to hold a stack of ultra-thin plates. A different number of ultra-thin plates are used according to the varied gap size as a result of the assembly in which parts having different dimensional tolerances are actually used.

Table 1: Embodiment 1

radius of curvature	space	refractive index	Abbe's No.	lens outer diameter
INF	0.46	1.5183	64.14	1
0.84	0.17	1		
INF	0.4	1.7323	54.68	1
-0.817	0.05	1		
1.353	0.65	1.7323	54.68	1
-0.703	0.25	1.7044	30.131	
-3.804	0.09	1		
INF (diaphragm)	0.03	1		
INF	0.4	1.5156	75.00	1
INF	0.2	1		
1.566	0.4	1.67	48.32	1
-1.566	0.2	1		
-0.729	0.3	1.5198	52.43	1
INF	0.56	1		
INF	0.4	1.5183	64.14	
INF	0.01	1.5119	63.00	
INF	0.4	1.6138	50.20	
INF	0.01	1.5220	63.00	
INF	0			

Distance to object 0 Image height 0.500

(Embodiment 2)

Fig.12 is a cross section of the lens of Embodiment 2 and Table 2 provides the lens data.

[0038]

The same reference numbers are given to the same components as in Embodiment 1 and their explanation is omitted.

Table 2: Embodiment 2

radius of curvature	space	refractive index	Abbe's No.	lens outer diameter
INF	0.88	1.8882	40.76	1.2
-0.703	0.05	1		
INF	0.4	1.5183	64.14	1.2
-1.485	0.05	1		
2.085	0.76	1.8081	46.57	1.2
-0.703	0.25	1.8126	25.42	1.2
INF	0.05	1		
INF (diaphragm)	0.03	1		
INF	0.4	1.5156	75.00	1.2
INF	0.43	1		
1.131	0.5	1.8395	42.72	1.2
-3.127	0.2	1		
-1.061	0.3	1.8126	25.42	1.2
INF	0.2	1		
-0.592	0.3	1.8081	46.57	1.2
2.132	0.77	1.8126	25.42	1.2
-1.262	0.77	1		
INF	0.4	1.5183	64.14	
INF	0.01	1.5119	63.00	
INF	0.4	1.6138	50.20	
INF	0.01	1.5220	63.00	
INF	0	1		

Distance to object 0 Image height 0.500

Table 3 provides the specification of the embodiments and Table 4 provides the values of the conditional expressions.

Table 3

item	legend	unit	Embodiment 1	Embodiment 2
scale factor of object system	β		-2.678847	-6.63
focal length of front group	f1	[mm]	0.765	0.591
focal length of rear group	f2	[mm]	3.476	4.557
focal length	f	[mm]	0.657	0.797
half of field angle	wy	[deg]	6.141	3.96
exit angle of main ray	wy'	[deg]	13.965	6.02
numerical aperture on theobject side	NA		0.2184	0.55
diaphragm diameter	ϕ1	[mm]	0.36	0.66
largest lens diameter	ϕ2	[mm]	1	1.2
pitch	P	[μ]	4	4
reference wavelength	λ	[μ]	0.546	0.546

Table 4

No.	Conditional expression	Embodiment 1	Embodiment 2
1	$1 < \mid\beta o\mid < 10$	2.680	6.630
2	$2 \leq f2 / f1 \leq 10$	4.544	7.711
3	$0.9 \leq \mid\cos wy' / \cos wy\mid \leq 1.1$	0.976	0.997
4	$0.1 \leq NA \leq 0.8$	0.220	0.550
5	$0.2 \leq \phi 1 / (\phi 2 * f1) \leq 2$	0.471	0.931
6	$0.1 \leq \mid 0.61 * \lambda * p / \beta o\mid \leq 0.8$	0.215	0.544

The video system suitable for *in vivo* cellular observation is described hereafter.

[0039]

As described above, living tissues consist of vascular parenchymal tissues and transparent epthelial cells overlying them. The following techniques are required to create the contrast between the cell nuclei and remaining portions that form the transparent cells and observe only the epithelial cells within a targeted observation region with no interference by the underlying parenchymal tissues.

[0040]

For example, the video system suitable for observing a layer of cells in question distinctly where cells are stained blue for contrast has the following configuration.

[0041]

Fig.3 shows a video system configuration.

The illumination light supplied by a light source 1 illuminates an object 3 via a video module 2. The light source 1 is provided with a wavelength selection filter 8, which is interposed in the illumination light path as required to produce illumination light having a wavelength profile suitable for cellular observation. When the illumination light having a visible wave range is used to illuminate an object, the shorter wavelength lights corresponding to the blue wave range reaches only the surface of a living tissue. This is useful to obtain information specific to the epithelial cells of the living tissue.

[0042]

The light around 500 nm that corresponds to the green wave range in the visible wave range reaches slightly deep below the surface of a living tissue. The light that corresponds to the red wave range in the visible

wave range reaches relatively deep inside a living tissue.

[0043]

An object image is formed on the image pick-up surface of an image pick-up element by an objective optical system of the magnifying video module 2. The object image is transformed into electric signals and sent to an image processing unit 4. In processing image data of the visible light range, green wavelength components are used to obtain the brightness of the object. This is to obtain the images close to those acquired through the human eye. The image data processed by the image processing unit 4 are displayed on a TV monitor 5.

[0044]

When the cells stained blue are illuminated by white light that equally contains blue, green, and red components, stained portions appear blue while unstained portions reflect the illumination light and appear white. Fig.4 shows a wavelength profile of a cellular image formed by the video module with the ordinate as light intensity (unit: arbitrary) and the abscissa as wavelength (unit: nm). Unstained portions do not absorb specific wavelength lights and give a nearly flat wavelength profile as is shown by the solid lines. Stained portions absorb the red light and give a wavelength profile with a drop in intensity at the red component as shown by the broken lines.

[0045]

In this way, the contrast between the background (unstained, white portions) and the cell nuclei and walls (stained, blue portions) is presented by the difference in absorbance of the larger wavelengths or red light within the visible wave range on images obtained by the video module.

[0046]

As shown in Fig.1, the epithelial cells are vertically layered. The images of layers of cells that are not at the depth of interest or of the underlying parenchymal tissues overlap with the background, reducing the image contrast. Cell walls are hardly stained compared to cell nuclei. Fig.6 shows an image where such image noises overlap the background and the cell walls are hardly recognizable.

[0047]

In order to eliminate the image noises overlapping the background, the light that transmits the information on the cell layers that are not at the depth of interest or on the parenchymal tissues can be cut off in the

illumination light path or in the objective optical system before the image pick-up element. In the embodiment of the video system, a filter for cutting off a specific wave range of wavelengths is inserted in the illumination light path to eliminate unnecessary wavelength components from the illumination light. In this case, unnecessary components are mainly the larger wavelengths in the visible light range such as the red light. However, it is not desirable to eliminate all the red light because this cuts off the wavelength components that serve to provide contrast between the stained, blue portions and the unstained portions.

[0048]

The present invention uses as the wavelength selection filter 8 for the light source a filter having a spectral transmittance shown in Fig.7. Fig. 7 shows the spectral transmittance with the ordinate as transmittance (unit: percent) and the abscissa as wavelength (unit: nm). More precisely, at least the illumination light consisting of the red wave range is divided into two wave bands R1 and R2 among the illumination light consisting the read, green, and red wave ranges and the wave band R1 closer to the green wave range is cut of by the filter. The wave band R1 ranges between 60nm and 700nm and the wave band R2 between 700nm and 800nm.

[0049]

Using the illumination light from which the wave band R1 closer to the green wave range is cut off allows limited light to reach cell layers that are not at the depth of interest or the parenchymal tissues within the depth of field of the objective optical system. The depth of field of the objective optical system is substantially reduced and the resolution in the depth direction is improved, thus preventing overlapping of images. On the other hand, the light in the wave band R2 contributes to producing contrast between the stained, blue cell nuclei and walls and the unstained remaining portions. Thus, a clear image of the cell layer of interest only is obtained with the information from unnecessary depths being eliminated. The characteristic shown in Fig.7 can be realized by a dichroic filter.

[0050]

An imaging agent can be used to enhance only the cell nuclei. In such a case, the imaging agent absorbed by the cell nuclei is excited by excitation light and emits fluorescent when it moves back to the ground state. This can be observed to precisely identify the cell nuclei. In particular, the video system according to the present invention is useful for a method where a gene imaging agent, a gene marker that reacts with light, (for example GFP) can be injected into the cells and the expression of a specific gene that occurs when healthy cells are transformed into the diseased such as cancer is identified.

[0051]

In the method above, the gene in a living cell is altered immediately before the onset of disease and a gene marker, which takes no action among the normal cells, is expressed and emits weak fluorescent in response to the excitation light. Thus, the video module used for this observation is desirably provided with a hypersensitive camera.

[0052]

It is preferred that the video system for fluorescent image observation of cell nuclei be used in combination with a conventional endoscope. The video module 2 is inserted into the body through the treatment tool insert of a conventional endoscope. Here, a conventional endoscope provides images for guiding the video module 2 to the targeted observation region. The TV monitor 5 displays both the image from the conventional endoscope and the fluorescent image from the video module image simultaneously. This allows the observation of the fluorescent image of cell nuclei along with the surrounding living tissue. This provides more precise and secure observation.

[0053]

When an imaging agent that primarily absorbs the light of wavelengths smaller than 480nm and emits the fluorescent of wavelength larger than 470nm is used, a preferred wavelength selection filter 8 for the light source has an apectral transmittance shown in Fig.9. In Fig.9, the spectral light transmittance is shown by the solid lines with the ordinate as transmittance (unit: percent) and the abscissa as wavelength (unit: nm).

[0054]

More precisely, the illumination light of the blue wave range is divided into two wave bands B1 and B2 and the wave band B1 closer to the green wave range is cut off by the filter. The wave band B1 ranges between 450nm and 500nm and the wave band B2 ranges between 350nm and 450nm. The objective optical system is provided with a filter that transmits the light of wavelengths larger than 470nm and cuts off the light of wavelengths smaller than 470nm. Thus, fluorescent images can be obtained while the excitation light is cut off. The light of the wave band B1 that includes the excitation and fluorescent wavelengths is cut off from the illumination light. This allows for clear images from weak fluorescent with no interference by the excitation light.

[0055]

The wavelength selection filter 8 for the light source can be accompanied with a filter that reduces the light intensity of the green or red wave range. This prevents unnecessary image noises on the background of

fluorescent images of the cell nuclei and allows a conventional endoscope to produce conventional observation images of a living tissue.

[0056]

The illumination method suitable for *in vivo* cellular observation is described hereafter with reference to Fig.13.

The tip of the illumination unit to illuminate an object and the tip of the objective optical system to form images on the image pick-up surface of an image pick-up element using the light from the object are located at the tip of an endoscope. The central axis of the illuminating field L is shifted from the central axis of the view field F by a distance d.

The endoscope is placed with the tip closely facing a living tissue to observe it. The distance x between the targeted region and the endoscope tip is adjusted so that the targeted region among the epithelial cells and parenchymal tissues constructing a living tissue is within the depth of field of the objective optical system. Here, it is assumed that the distance between the epithelial cells and endoscope tip is X1 and the distance between the parenchymal tissues and endoscope tip is X2.

[0057]

The conventional endoscope uses the view field F2 of the objective optical system to observe the parenchymal tissues at a distance of X2. The distance d between the central axes of the illuminating field L and view field F at the endoscope tip is determined in the manner that the illuminating field L2, which is ahead by a distance of X2, includes the view field F2 in order to ensure uniform brightness in the view field F2. The distance X2 is several millimeters to several tens millimeters.

[0058]

On the other hand, the magnifying endoscope uses the view field F1 of the objective optical system to observe the epithelial cells at a distance of X1. The distance X1 is zero to several microns. Here, the distance between the endoscope tip and object is hardly reserved. Therefore, the illuminating field L1a, which is ahead by the distance X1, fails to include the view field F1 even if the distance d between the central axes of the illuminating field L and view field F at the endoscope tip is reduced. Consequently, it is understood that the conventional illumination method fails to ensure uniform brightness in the view field F1 of the objective optical system. The present invention provides an illumination method in which the parenchymal tissues that is further ahead of the depth of field is used to ensure uniform brightness in the view field F1.

[0059]

In Fig.13, an observation target is at a distance of X1 from the endoscope tip and the parenchymal tissues are at a distance of X2. The illumination light emitted from the endoscope tip reaches the parenchymal tissues via the illuminating field L1a. The parenchymal tissues serve as a reflecting and scattering surface to scatter the illumination light. It is assumed that the illumination light emitted from the endoscope tip has a light distribution profile according to Gaussian distribution and the useful light distribution angle of illumination is defined as a light distribution angle ω that provides the light intensity 1/e (e is the root of logarithm natural) in relation to the light intensity for the light distribution angle of zero.

[0060]

The illumination light emitted from the endoscope tip is transmitted through the living tissues at the light distribution angle ω1' before it reaches the parenchymal tissues. Reflected and scattered by the parenchymal tissues, the illumination light is transmitted through the living tissues at the light distribution angle ω2' before it reaches the epithelial cells and forms the illuminating field L1b at a distance X1 from the endoscope tip that includes the view field F1 of the objective optical system. Consequently, uniform brightness is ensured in the view field F1 of the objective optical system.

[0061]

ω1' and ω2' are light distribution angles in living tissues. The following expressions are used to convert them to those in the air.

$$\sin \omega 1 = 1.33 \times \sin \omega 1'$$
$$\sin \omega 2 = 1.33 \times \sin \omega 2'$$

The parenchymal tissues are outside the depth of field of the objective optical system; therefore, the light reflected and scattered by the parenchymal tissues is not imaged and merely the illumination effect on the epithelial cells is obtained. It is preferred that the following condition be satisfied for the distance d between the central axes of the illuminating field L and view field F at the endoscope tip:

[0062]

$$1 \leq \log (d / X1 \times \tan \omega)) \leq 3 \ldots (10)$$

When the conditional expression exceeds the upper limit, uniform brightness will not be ensured in the view field of the objective optical system. When the conditional expression exceeds the lower limit, it will be difficult to locate the tips of the illumination unit and objective optical system at the endoscope tip with the

outer diameter thereof being maintained small.

[0063]

In addition, the following condition is preferably satisfied:

$5 \leq (d / X2 \times \tan \omega)) \leq 30$ ... (12)

When the conditional expression exceeds the upper or lower limit, uniform brightness will not be ensured in the view field of the objective optical system.

[0064]

It is desired that the distance X1 between the leading surface of the endoscope and the in-focus point of the objective optical system and the distance X2 between the leading surface of the endoscope and the reflecting and scattering surface, such as the parenchymal tissues, satisfy the following relationship:

[0065]

$0.5 \leq \log (X2 / X1)$ ... (11)

When the conditional expression exceeds the lower limit, the parenchymal tissues are imaged in the view field, deteriorating the image quality.

[0066]

Table 5 provides the specification of the embodiments of the illumination method according to the present invention and Table 6 provides the values of the conditional expressions.

Table 5

item	legend	unit	Embodiment 1	Embodiment 2
scatterer distance	X2	[mm]	0.1	0.1
objective in-focus distance	X1	[mm]	0.015	0.002
illumination parallax	d	[mm]	0.8	1
illumination distribution angle	ω	[deg]	35	35

Table 6

No.	Conditional expression	Embodiment 1	Embodiment 2
10	$1 \leq \log (d / X1 \times \tan \omega)) \leq 3$	1.88	2.85
11	$0.5 \leq \log (X2 / X1)$	0.82	1.7
12	$5 \leq (d / X2 \times \tan \omega)) \leq 30$	11.4	14.3

The method for diagnosing the presence/absence of abnormal cells (whether cells are cancerous or not) from magnified cell images is described hereafter.

[0067]

Fig.5 shows stained cells that are magnified and observed according to Embodiment 1. The video module used in the magnifying video system is set for a scale factor that allows several tens to several hundreds of nuclei to be observed on a monitor. For example, several tens to several hundreds of cell nuclei are displayed on a monitor and the cell density in the observation view field can be evaluated based on the distance between cell nuclei so as to diagnosis the presence/absence of abnormal cells. The cell density can be compared with normal samples and statically analysed.

[0068]

The magnifying video system above is specified for resolution and view range sufficient for nucleic observation.

With the observation scale factor further increased, the video module displays several cell nuclei on a monitor where the number of cell nuclei in a unit area is translated to the cell size or the cell nuclei are evaluated for shape to diagnosis the presence/absence of abnormal cells. For example, cancerous cells present particular characteristics such as increased sizes and irregular shapes. The size and shape of cell nuclei can be evaluated to diagnosis cancerous cells.

[0069]

Fig.6 shows cell nuclei and cell walls that are magnified and observed according to Embodiment 2. The magnifying video system is set for a scale factor that allows several nuclei to be observed on a monitor. The area S of cell walls and the area S' of nuclei are used to calculate the occupancy of nuclei in cells. The occupancy is used to diagnosis the presence/absence of abnormal cells. The magnifying video system like this is specified for resolution and contrast sufficient for observation of both cell nuclei and cell walls.

[0070]

Fig. 14 is a flowchart of a series of procedures for *in vivo* cellular observation described above.
The system described above in which specific wave bands are filtered off from the white light source provides an excellent flexibility where plural wave properties are selectively used in the illumination light depending on the observation target and selected coloring agent. On the other hand, where the wavelength

property of the illumination light is predetermined, for example a single color illumination light can be used to further simplify the configuration.

[0071]

An imaging system specified for observation with specific wavelengths is illustrated in Fig.10. The video module according to the present invention has a small observation distance and, therefore, a light source 17 can be one that emits a single color in low power, such as a LED. The LED light source can be mounted on the endoscope body. Furthermore, an illumination transmission fiber can be eliminated when the LED light source is installed at the tip. An observation system 11 can be provided with a simplified optical system 13 because aberration correction is necessary only for a single color. For example, a single aspherical lens can be placed in front of a diaphragm 14 or plural spherical lenses can be used in place of an aspherical lens.

[0072]

The video module down-sized and simplified as described above has more freedom in layouts to be mounted on medical devices such as endoscopes. For example, it can be combined with a treatment tool such as a catheter or laser probe with a flexible type insertable device and it can take a compact shape such as a pen or capsule shape by using wireless transmission of image data with a hard type insertable device.

[0073]

[Efficacy of the invention]

The magnifying video system, endoscope, and observation method using them as described above realize a real-time *in vivo* cellular observation.

[Brief description of the drawings]

[Fig.1]

Fig.1 is an illustration to explain the principle of living tissue observation using a prior art endoscope.

[Fig.2]

Fig.2 is an illustration to explain the principle of sample observation using a microscope.

[Fig.3]

Fig.3 is an illustration to show the configuration of the video system according to the present invention.

[Fig.4]

Fig.4 is a graphical representation to show the wavelength distribution property of an image formed by the video module according to the present invention.

[Fig.5]

Fig.5 is an illustration to show stained cells magnified and observed according to an embodiment of the present invention.

[Fig.6]

Fig.6 is an illustration to show an image in which the underlying cells overlap the background.

[Fig.7]

Fig.7 is a graphical representation to show the spectral transmittance of a wavelength selection filter used in an embodiment of the present invention.

[Fig.8]

Fig.8 is an illustration to show an image in which information at unnecessary depth is eliminated on the contrary to the image in Fig.6.

[Fig.9]

Fig.9 is a graphical representation to show the spectral transmittance of another wavelength selection filter used in an embodiment of the present invention.

[Fig.10]

Fig.10 is an illustration to show an embodiment of an imaging system specified for observation with specific wavelengths.

[Fig.11]

Fig.11 is a cross section of the lens of Embodiment 1 of the present invention.

[Fig.12]

Fig.12 is a cross section of the lens of Embodiment 2 of the present invention.

[Fig.13]

Fig.13 is an illustration to show the illumination method suitable for *in vivo* cellular observation.

[Fig.14]

Fig.14 is a flowchart of procedures of *in vivo* cellular observation according to the present invention.

[Legend]

1, 17	light source
2	video module
3	object
4	image processing unit
5	TV monitor
8	wavelength selection filter
11	observation system
13	optical system
14	diaphragm
101	objective lens group
102	objective frame
103	aperture diaphragm
104	cover glass
105	image pick-up element
106	image pick-up frame
107	space
108	hard tip
109	intermediate member
110	sheath
111	illumination fiber
112a, 112b	gap adjustment part
L1a, L1b, L2	illuminating field
F1, F2	view field

[Title of the document] Figure

[Fig.1]

endoscope tip part
mucus
cell wall diffraction
cell nucleus
epithelial cells
parenchymal tissues
blood vessel

[Fig.2]

microscope observation system
sample
cover glass
illumination system

[Fig.3]

(5) TV monitor

[Fig.4]

solid line   unstained portion -> white
broken line   stained portion -> blue
difference in absorbance of stained cell

[Fig.5]

[Fig.6]

[Fig.7]

differences in absorbance wavelength seen for larger wavelengths.

[Fig.8]

[Fig.9]

differences in absorbance wavelength seen for smaller wavelengths.

[Fig.10]

(a) insert tip part

[Fig.11]

[Fig.12]

[Fig.13]

illumination system observation system

endoscope tip

in-focus position

illuminating field L1a, view field F1, illuminating field L1b

scattering surface (parenchymal tissues)

illuminating field L2, view field F2

[Fig.14]

pre-treatment

detect suspicious region by conventional endoscope
deliver coloring agent under conventional endoscope
cells are selectively stained due to difference in time for cells to intake/excrete coloring agent
guiding magnifying endoscope to suspicious region and contact and fix it thereto under conventional endoscope

cell visualization

emit illumination light having selected wavelengths
wave band T2:
visualize cells due to difference in absorbency
wave band T1:
cut off data at depths not targeted

abnormal cells diagnosis

display several tens to several hundreds of cell nuclei
display several cell nuclei and cell walls

[Title of the document] Abstract

[Abstract]

[Purpose]

To provide a video system for realizing real-time *in vivo* cellular observation, an *in vivo* cellular observation method using the video system, and an endoscope for *in vivo* cellular observation.

[Resolution means]

A video system provided with a video module 2 comprising an objective optical system having a scale factor larger than 1 and an illumination optical system and with a light source for supplying illumination light, characterized by the fact that the light source 1 is provided with a wavelength selection means 8 for dividing, among the blue, green, and red wave ranges in the illumination light, the blue or red wave range into two wave bands T1 and T2 and cutting off the wave band T1 closer to the green wave range.

[Selected figure] Fig.3